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Effect of External Electric Field Upon Selected Proteogenic Amino Acids

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Abstract
Effect of external electric field (EEF) of 0.001, 0.005 and 0.01 a.u. upon molecular energy, charge distribution and dipole moments of non-dissociated and inner salt forms of alanine (Ala), arginine (Arg), aspartic acid Asp), cysteine (Cys), glutamic acid (Gln), glycine (Gly), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), ornithine (Orn), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr), and valine (Val) were studied. For that purpose HyperChem 8.0 software was used together with the AM1 method for optimization of the conformation of the molecules in a computer vacuum. Based on the effect of EEF upon the charge density localized at the nitrogen atom of the α-amino group the acids were divided into two groups. They were Group I in which EEF increased the negative charge (Ala, Gly, Ile, Leu, Lys, Met, Phe, Pro, and Thr) and Group II in which EEF induced opposite effect (Cys, Ser, Tyr and Val). Generally, an increase in the EEF strength declined energy and increased dipole moments in non-ionized amino acids and in their inner salt forms. Energy of non-dissociated forms was more negative than these of corresponding zwitterions. Orientation of the molecules in EEF strongly depended on the EEF strength.

Key words: Alanine; Arginine; Aspartic acid; Cysteine; Glutamic acid; Glycine; Isoleucine; Leucine; Lysine; Methionine; Ornithine; Proline; Serine; Threonine; Tryptophan; Tyrosine; Valine

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INTRODUCTION
Endogenous electric field controls organization of the living organisms (Pokorny, Hasek, & Jelinek, 2005). The existence of that field strongly implies an essential effect of external electric field (EEF) upon living matter. Indeed, there are numerous reports documenting such effect. Living cells absorb and convert energy of external electric field (EEF) inducing active transport of K^+ and Ca^{2+} ions through the membranes and ATP synthesis involving ATP-synthetizes (Tsong & Astumray, 1986). That results in perturbation of active transport of ions and ATP synthesis as well as changes conformation of enzymes. Conformational changes were observed in *Saccharomyces cerevisiae* under the influence of pulsed EEF (Harrison, Barbosa-Canovas, & Swanson, 1997). Experiments with supersaturated aqueous glycine solutions pointed to nucleation of γ-polymorphs (Aber, Arnold, & Garetz, 2005) and such processes can take place in living cells exposed to EEF. Thus, EEF can regulate metabolism of alcohol (Crabb, Bosron, & Li, 1987; Ambroziak & Pietruszko, 1993; Berry, Grivel, & Phillips, 1993). Pulsed EEF stimulated microbial production of ethanol (Grosse, Bauer, & Berg, 1988; Nakanishi, Tokuda, Soga, Yoshinaga, & Takeda, 1988). It stimulated also synthesis of ADNP in respiration inhibited submitochondrial particles (Tiessie, Knox, Tsong, & Wehrle, 1981), citric acid production by *Aspergillus niger* (Fiedurek, 1999), and switching of the elevated enzymatic reactions in micelles (Harada & Kataoka, 2003). The EEF influenced also the plant growth under microgravity conditions (Nechitailo & Gordeev, 2001). Pulsed EEF was considered as the factor controlling the growth and activity of *Escherichia coli* and *Listeria innocua* in liquid food products (Dutreux et al., 2000). Papers cited above together with numerous reports on the subject describe the phenomenon and interpret it on the macromolecular level. This paper describes behavior of selected proteogenic α-amino acids placed in EEF of increasing strength. This study should be understood

as introduction to understanding effect of EEF upon peptides, polypeptides and proteins as well as possibility of carrying some EEF stimulated reactions of amino acids. The applied approach was utilized in our former papers for the presentation of the effect of EEF upon simple gaseous molecules (Mazurkiewicz & Tomasik, 2010), selected monosaccharides (Mazurkiewicz & Tomasik, 2012a), lower alkanols (Mazurkiewicz & Tomasik, 2012b), porphin and metalloporphyrins (Mazurkiewicz & Tomasik, in press).

Obviously, carboxylic and amino groups of proteogenic (biogenic) α-amino acids taken under consideration in this project dispose with acidic and basic centers, respectively. Thus, these amino acids spread into these neutral having one amino and one carboxylic group, but these with additional centers of acidity such as thiol SH (cysteine), phenolic OH (tyrosine) and indol NH are acidic. Basic acids contain two amino groups and one carboxylic group. Acidic amino acids in addition to these mentioned above have two acidic and one basic centers. In the neutral aqueous solution as well as in the solid state they exist in the form of inner salts formed as a consequence of intramolecular transfer of the proton of the carboxylic group onto the amino group. In acidic amino acids the carboxylic group closer to the amino group is more dissociated and participates in the formation of the inner salt. In basic amino acids the more basic amino group residing on the longer distance from the carboxylic group is more basic than the α-amino group and, therefore, it accepts the carboxylic proton (Jakubke & Jeschkeit, 1973).

This paper presents effect of EEF of 0.001, 0.005 and 0.01 a.u. upon selected non-ionized forms and inner salts of proteogenic amino acids that is alanine (Ala), arginine (Arg), asparagic (aspartic) acid (Asp), cysteine (Cys), glutamic acid (Glu), glycine (Gly), leucine (Leu),), lysine (Lys), methionine (Met), ornithine (Orn), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr) and valine (Val). The selected acids are these which the most commonly are encountered in proteins. These effects of EEF are given in terms of numerically computed changes in the charge distribution, dipole moments and simulated changes of conformations and/or orientation along the field.

1. COMPUTATIONS

HyperChem 8.0 software was used together with the AM1 method for optimization of the conformation of the molecules of amino acids under study. Then, charge distribution, potential and dipole moment for molecules placed in the external electric field of 0.000, 0.001, and 0.01 a.u. were calculated. The molecules were situated along the x-axis. The y- and z-axes were perpendicular in plane and perpendicular to plane containing this structure, respectively.

2. RESULTS AND DISCUSSION

Total energy of the non-dissociated amino acids and their inner salt forms, obviously, decreases with the increase in the number of the atoms in the molecules. That energy depends also on the conformation of the molecules as shown in Table 1. Except Cys, energy of the inner salts are higher, that is, less negative than energy of non-dissociated acids. Energy of the non-dissociated as well as inner salts of every amino acid slightly decreases with increase in the strength of applied field. Non-dissociated molecules and their inner salts distinguish in their sensitivity to EEF. As the strength of EEF increases the gap between energy of both forms gradually ceases and in case of glutamic acid the energy of the inner salt in the EEF of 0.01 a.u. is less negative than that of the non-dissociated acid placed in the same field. Generally, the reduction of the gap due to increase in EEF from 0.000 to 0.0 a/u. reaches from, approximately, 50 to 66% of original value.

Table 1
Total Energy [kcal/mole and [a.u.] of Non-dissociated Amino Acids and Their Inner Salts Placed in External Electric Field (EEF) **of Varying Strength [a.u.]**[a,b]

Amino acid and number of its atoms	Total energy at EEF [a.u.]							
	0.000		0.001		0.005		0.01	
	kcal/mole	a.u.	kcal/mole	a.u.	kcal/mole	a.u.	kcal/mole	a.u.
Monoamino monoic acids								
Gly 10	-24545.3 *-24509.6* *(35.7)*	-39.1	-24546.0 *-24512.5* *(33.5)*	-39.1	-24549.1 *-24524.4* *(24.6)*	-39.1	-24553.4 *-24540.4* *(13.0)*	-39.1
Ala 13	-27993.9 *-27965.5* *(28.3)*	-44.6	-27995.0 *-27968.0* *(26.9)*	-44.6	-27999.8 *-27978.0* *(21.7)*	-44.6	-28007.2 *-27993.0* *(14.2)*	-44.6
Cys 14[c]	-32280.2 *-36116.8*	-51.4	-32280.9 *-36117.8*	-51.4	-32284.8 *-36125.0*	-51.4	-32291.1 *-36139.6*	-51.5
Pro 17	-34172.3 *-34141.7* *(30.5)*	-54.5	-34173.6 *-34144,4* *(29.2)*	-54.5	-34178.1 *-34155.7* *(22.4)*	-54.5	-34185.1 *-34170.9* *(14.2)*	-54.5

To be continued

Continued

Amino acid and number of its atoms	Total energy at EEF [a.u.]							
	0.000		0.001		0.005		0.01	
	kcal/mole	a.u.	kcal/mole	a.u.	kcal/mole	a.u.	kcal/mole	a.u.
Ser 14	-34765.2 *-34734.6* *(30.5)*	-55.4	34765.4 *-34737.2* *(28.1)*	-55.4	-34769.9 *-34750.6* *(19.3)*	-55.4	-34773.9 *-34765.8* *(10.1)*	-55.4
Val 19	-34889.3 *-34860.8* *(28.4)*	-55.6	-34888.3 *-34863.3* *(25,0)*	-55.6	-34895.2 *-34874.0* *(21.1)*	-55.6	-34902.5 *-34888.3* *(14.1)*	-55.6
Thr 17	-38214.1 *-38188.4* *(25.7)*	-60.9	-38214.6 *-38190.7* *(23.9)*	-60.9	-38217.4 *-38200.5* *(16.9)*	-60.9	-38221.7 *-38214.4* *(7.3)*	-60.9
Leu 22	-38337.3 *-38304.0* *(33.2)*	-61.1	-38339. 9 *-38306.7* *(33.1)*	-61.1	-38342.8 *-38318.2* *(24.5)*	-61.1	-38347.4 *-38333.7* *(13.7)*	-61.1
Ile 22	-38339.4 *-38306.3* *(33.0)*	-61.1	-38339.5 *-38309.0* *(30.4)*	-61.1	-38341.4 *-38320.5* *(20.8)*	-61.1	-38344.6 *-38336.2* *(8.3)*	-61.1
Met 20	-39175.6 *-39146.0* *(29.5)*	-62.4	-39177.8 *-39152.4* *(25.4)*	-62.4	-39184. 0 *-39161.9* *(22.0)*	-62.4	-39193.4 *-39176.0* *(17.3)*	-62.5
Phe 23	-45797.5 *-45765.3* *(32.1)*	-73.0	-45798.1 *-45768.1* *(29.9)*	-73.0	-45801.9 *-45780.5* *(21.4)*	-73.0	-45808.2 *-45798.2* *(10.0)*	-73.0
Tyr 24	-52573.7 *-52546.7* *(26.9)*	-83.8	-52574.4 *-52549.4* *(24.9)*	-83.8	-52579.4 *-52561.3* *(18.0)*	-83.8	-52587.5 *-52578.5* *(9.2)*	-83.8
Diamino monoic acids								
Orn 21	-38986.2 *-38910.4* *(75.8)*	-62.1	-38986.7 *-38917.3* *(69 4)*	-62.1	-38989.6 *-38946.0* *(43.5)*	-62.1	-389940 *-38983.4* *(10.5)*	-62.1
Lys 24	-42433.8 *-42399.9* *(33.9)*	-67.6	-42434.5 *-42401.4* *(33.1)*	-67.6	-42438.6 *-42418.9* *(19.7)*	-67.6	-42446.9 *-42437.5* *(9.4)*	-67.6
His 20	-44366.9 *-44365.9* *(0.9)*	-70.7	-44368.5 *-44366.6* *(1.8)*	-70.7	-44376.3 *-44369.9* *(6.4)*	-70.7	-44388.1 *-44383.5* *(4.6)*	-70.7
Arg 26	-49903.8 *-49880,6* *(23.2)*	-79.5	-49904.7 *-49887.7* *(13.9)*	-79.5	-49909.8 *-49898.6* *(11.1)*	-79.5	-49923.5 *-49914.5* *(8.8)*	-79.6
Trp 27	-55353.6 *-55320.1* *(33.4)*	-88.2	-55353.6 *-55328.7* *(24.8)*	-88.2	-55359.6 *-55341.8* *(17 .7)*	-88.2	-55369.4 *-55361.0* *(8.4)*	-88.2
Monoamino dioic acids								
Asp 16	-44283.2 *-44252.7* *(30.5)*	-70.6	-44284.2 *-44254.1* *(30.1)*	-70.6	-44288.9 *-44260.6* *(28.2)*	-70.6	-44295.8 *-44270.1* *(25.7)*	-70.6
Glu 19	-47725.6 *-47709.2* *(16.3)*	-76.1	-47727.2 *-47711.9* *(15.2)*	-76.1	-47734.5 *-47728.3* *(6.2)*	-76.1	-47745.9 *-47747.1* *(-1.2)*	-76.1

[a]Lower cases in italics are related to inner salts.
[b]In parentheses the energy gap between values for non-dissociated acid (E_{nd}) and its inner salt (E_{is}), that is, $E_{nd} - E_{is}$, is given.
[c]The applied computation method indicated anomalously long bond between the carbonyl group and the α-carbon atom of the chain in the inner salt structure.
Therefore, the results of the computations for the acid inner salt are not credible.

In non-dissociated amino acids EEF causes changes in the dipole moment of the amino acid molecules. Except Val and Leu, as a rule, a total dipole moments increase with the EEF strength. However, its components along the axes of the Cartesian systems vary irregularly (Table 2) as the molecules orient in the field in a diverse manner, depending on their structure and the field strength. In case of Val and Leu their total dipole moment in EEF of 0.001 a.u. is lower than that for the molecule out of EEF. In Val, dipole moment along the x-axis is larger than that in the 0.001 a.u. field whereas the component along z-axis is smaller when the molecule is out of the field. In EEF of 0.001 a.u. conformation taken by that molecule can provide interaction of one of the hydrogen atoms of the

methyl groups with the carbonyl oxygen atom and/or the amino group nitrogen atom Such interaction decreasing dipole moment is known in such kind molecules and quantitatively treated as so-called Hancock Δ6 number (Hancock, Yager, Falls, & Schreck, 1963). In Leu, dipole moment along z-axis significantly changes at EEF of 0.001 a.u. obviously, dipole moments for inner salts are essentially higher that these for non-dissociated molecules. With some exceptions, they also increase with increase in the EEF strength. Some irregularities are met in case of inner salts of Met exposed to EEF of 0.001 a.u., Tyr in the EEF of 0.005 a.u. and Arg for which dipole moment in any EEF is lower than that for the molecule out of that field.

Table 2
Dipole Moments [D] of Amino Acids Along x, y and z Axes of the Cartesian System and Total Dipole Moments[a]

Amino acid	Dipole moments [D] at EEF [a.u.]															
	0.000				0.001				0.005				0.01			
	x	y	z	Total	x	y	z	Total	x	y	z	Total	x	y	z	Total
Monoamino monoic acids																
Gly	1.142	-1.014	2.362	2.813 *11.49*	-2.887	-0.246	0.228	2.907 *11.69*	-3.291	-0.044	0.042	3.291 *12.40*	-3.761	-0.016	0.029	3.761 *13.20*
Ala	3.554	-2.272	-1.089	4.357 *10.27*	-4.505	-0.463	0.223	4.534 *10.45*	-5.237	-0.010	0.063	5.238 *11.15*	-6.440	-0.030	0.028	6.440 *11.99*
Cys	2.759	-0.544	-1.020	2.991 *3.97*	-3.248	-0.396	-0.378	3.294 *4.49*	-4.446	-0.087	-0.045	4.447 *8.64*	-5.752	-0.016	-0.037	5.753 *11.29*
Pro	3.078	-0.887	1.586	3.574 *10.72*	-3.710	-0.624	0.462	3.791 *10.94*	-5.146	-0.088	0.044	5.147 *11.82*	-6.175	-0.047	-0.002	6.175 *12.90*
Ser	-0.596	-0.074	-0.481	0.769 *10.33*	-0.827	-0.029	-0.491	0.962 *10.60*	-2.703	0.018	-0.069	2.704 *11.85*	-3.747	0.001	-0.035	3.748 *12.76*
Val	4.110	0.327	-0.618	4.169 *10.12*	3.874	0.241	-0.751	3.953 *10.33*	-5.378	0.082	-0.070	5.379 *11.15*	-6.451	0.027	-0.030	6.451 *12.15*
Thr	-0.590	0.878	-2.004	2.266 *9.22*	-2.274	0.310	-0.760	2.418 *9.43*	-2.274	0.310	-0.760	2.418 *10.28*	-3.874	-0.005	-0.040	3.874 *11.67*
Leu	-2.198	-0.665	-1.612	2.806 *10.85*	-2.373	-0.509	0.462	2.470 *11.10*	-3.276	-0.132	0.114	3.281 *12.03*	-4.251	-0.043	0.045	4.252 *13.11*
Ile	0.169	-0.679	0.934	1.167 *10.87*	-0.407	-0.685	0.915	1.214 *11.14*	-2.068	-0.055	0.151	2.074 *11.15*	-3.034	0.031	0.081	3.035 *13.29*
Met	4.049	3.038	-0.092	5.063 *9.50*	-5.539	0.952	-0.073	5.620 *9.01*	-6.834	0.135	0.051	6.836 *10.13*	-8.338	0.041	0.047	8.339 *11.92*
Phe	2.581	-0.668	1.162	2.909 *11.31*	-2.607	0.572	1.574	3.098 *11.73*	-4.294	0.123	0.242	4.303 *13.33*	-5.837	0.049	0.089	5.838 *15.22*
Tyr	0.877	-3.095	1.762	3.667 *10.77*	-3.090	-2.352	0.623	3.933 *11.18*	-5.607	-0.253	0.133	5.614 *14.86*	-7.589	-0.071	0.018	7.589 *14.98*
Diamino monoic acids																
Orn	0.628	0.276	2.126	2.234 *28.15*	-2.049	-0.772	0.980	2.399 *28.46*	-3.136	-0.147	0.068	3.140 *29.58*	-4.012	-0.079	0.043	4.013 *30.09*
Lys	1.983	-2.379	-0.581	3.151 *9.47*	-3.060	-1.362	-0.757	3.434 *9.86*	-4.683	-0.155	-0.131	4.688 *10.25*	-6.339	0.040	0.101	6.340 *11.75*
His	0.169	-0.679	0.934	1.167 *2.64*	-6.588	-1.341	0.378	6.734 *2.84*	-8.711	-0.132	0.044	8.712 *3.77*	-10.261	-0.057	0.018	10.262 *7.72*
Arg	0.488	2.874	1.153	3.153 *13.29*	-3.599	1.020	-0.871	3.841 *10.28*	-6.143	-0.089	-0.139	6.145 *11.86*	-10.570	-0.057	-0.085	10.571 *11.86*
Trp	-4.800	-0.396	-1.315	4.993 *8.70*	-4.801	-0.396	-1.315	4.993 *12.23*	-6.867	-0.171	-0.093	6.869 *14.33*	-9.062	-0.078	-0.013	9.062 *16.69*
Monoamino dioic acids																
Asp	-0.952	-3.214	2.285	4.057 *5.79*	-4.174	-0.754	0.160	4.245 *6.02*	-5.086	-0.115	0.018	5.087 *7.04*	-6.196	-0.058	-0.004	6.196 *8.37*
Glu	2.838	-5.166	-2.690	6.479 *9.30*	-6.713	-0.852	0.444	6.782 *12.10*	-8.019	-0.132	0.066	8.021 *14.60*	-9.784	-0.084	0.027	9.784 15.89

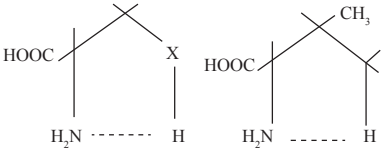
[a]Lower cases in italics are related to inner salts

Particular amino acids and their inner salts distinguish from one another in the sensitivity to the changes of dipole moments under the influence of EEF. One may see that dipole moment vs. EEF dependences for majority of non-dissociated acids is linear and they have very similar slope (Figure not shown). Exclusive behavior exhibit these for Arg which is very sloppy, His which anomalously increases in the region between 0.000 and 0.001 a.u., Thr which is concave up and Ser which is S-shaped. In case of Val and Leu application of EEF of 0.001 a.u. produces a slight decrease in dipole moments of these molecules. It can be rationalized in terms of certain changes in the conformation of the molecules.

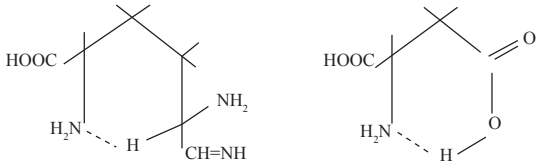
Similarly, majority of such relationships for inner salts are linear and fairly flat. Only functions for Cys and His are S-shaped, for Lys and Thr are slightly concave up and these for Trp, Glu and Tyr anomalously increase in the initial stage. Dipole moments of Met and Arg decline after application of EEF of 0.001 a.u. Such decrease is particularly strong for Arg (Figure not shown).

In the group of non-dissociated monoamino monoic acids, as a rule, increase in the charge density results in increase in the negative charge density on the oxygen atom of the carboxyl group and in positive charge on the carbonyl carbon atoms. These densities vary fairly regularly with the applied EEF strength. The effect of EEF on the charge density on the nitrogen atom of the α-amino group allows to spread amino acids under consideration into these in which negative charge density decreases with the EEF strength (Ala, Gly, Ile, Leu, Lys, Met, Phe, Pro, and Thr) (Group I) and these in which the charge density varies opposite way (Cys, Ser, Tyr and Val) (Group II). Moreover, the changes in the charge density at that atom vary sometimes irregularly with EEF. In the inner salt molecules increase in the EEF strength produces decrease in the positive charge of the quarternized amino group in Gly, Cys, Ser, Leu, Ile, Met, Phe, Asp and Glu acid. In Ala, Pro,Val, Thr, Tyr, Orn, Lys, His, Arg and Trp positive charge of that group increases. Thus, the reaction of the charge density at the protonated nitrogen atom of the α-amino group is in no relation to accounting non-dissociated amino acid to either Group I or Group II. The Group II of amino acids is constituted by these acids which in their side chains posses either SH, OH, COOH or methylene groups, the latter activated by the negative inductive effect of vicinal substituent.

These molecules can take thermodynamically favored semi-cyclic conformations stabilized by intramolecular interactions as presented by structures for Cys (**1**), Ser (**2**), Glu acid (**3**), Val (**4**), Tyr, Arg and Asp, respectively. Cys, Ser and Glu acid can take conformations stabilized with intramolecular hydrogen bond providing five-membered rings whereas Val, Tyr, Arg and Asp take conformation providing six-membered rings.



1: X = S; Cys **4** **5**
2: X = OH; Ser
3: X = CH-COOH; Glu acid



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Although Thr also possesses β-hydroxyl group in its structure, it belongs to the Group I amino acid, likely because of conformations taken in EEF which do not favor formation of intramolecular N…H-O hydrogen bond. Since charge density on hydrogen atoms of the methyl groups in Val and Thr are comparable (see Supplementary Material) steric reasons seem to be involved.

Sensitivity of the charge densities at particular atoms of the amino acids to increase in the EEF strength depend on the structure of the conformational changes and hence localization of the molecules in EEF. Comparison of the negative charge density at the amino nitrogen atom, in Gly and Ala, essentially more negative in Ala, reflects the inductive effect of the methyl group. The inductive effect of the methyl group is expressed by Taft substituent constant $\sigma^* = 0.000$ compared to $\sigma^* = 0.490$ (Taft, 1953) for the hydrogen atoms in Gly. The case of Arg illustrates the role of the conformation and situating the molecule in EEF. The negative charge at the α-amino group nitrogen atom varies irregularly with increase in the EEF strength. Depending on the latter, Arg belongs first to the Group I amino acid and then shifts to Group II.

Results of computations of the charge densities at particular atoms of amino acids under consideration are available in supplementary material.

CONCLUSION

External electric field (EEF) influences α-amino acids regardless they take the non-ionized or inner salt structure. The effect becomes more pronounced with increase in the EEF strength and is more remarkable in the inner salts. That influence is reflected by increase in dipole moment of the molecules and electron density distribution in the molecules. These effects can perturb biological functions of the amino acid condensates.

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APPENDIXES

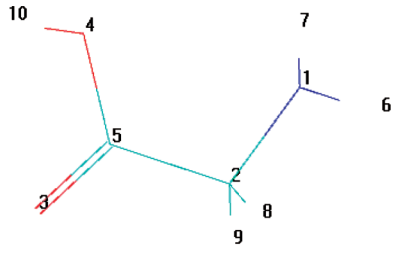

Figure 1
Atom Numbering in the Gly Molecule

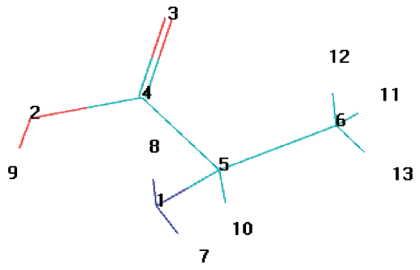

Figure 2
Atom Numbering in the Ala Molecule

Table 1
Charge Density at Particular Atoms of the Gly Molecule Varying With the EEF Strength[a,b]

EEF a.u.	Atom									
	1	2	3	4	5	6	7	8	9	10
0.000	-.017 *.902*	-.095 *-.504*	-.384 *-.474*	-.311 *-.609*	.380 *.479*	.031 *.014*	.035 *.014*	.052 *.112*	.081 *.112*	.229 *-.046*
0.001	-.019 *.899*	-.095 *-.493*	-.391 *-.483*	-.308 *-.610*	.382 *.476*	.033 *.014*	.036 *.014*	.068 *.112*	.080 *.112*	.227 *-.041*
0.005	-.028 *.893*	-.095 *-.459*	-.420 *-.516*	-.295 *-.614*	.390 *.466*	.041 *.015*	.041 *.015*	.068 *.112*	.076 *.112*	.221 *-.025*
0.01	-.038 *.890*	-.095 *-.428*	-.457 *-.555*	-.278 *-.617*	.401 *.458*	.050 *.015*	.048 *.015*	.083 *.113*	.072 *.113*	.214 *-.006*

[a]In the inner salt atom 10 moves to atom 1.
[b]Upper cases and lower cases in italics are related to non-dissociated acid and its inner salt, respectively

Table 2
Charge Density at Particular Atoms of the Ala Molecule Varying With the EEF Strength[a,b]

EEF [a.u.]	Atom												
	1	2	3	4	5	6	7	8	9	10	11	12	13
0.000	-.044 *.669*	-.266 *-.583*	-.340 *-.451*	.355 *.448*	-.114 *-.374*	-.133 *-.103*	.040 *-.008*	.039 *-.001*	.209 *.116*	.086 *.112*	.068 *.029*	.056 *.054*	.044 *.092*
0.001	-.045 *.673*	-.269 *-.585*	-.348 *-.460*	.358 *.447*	-.114 *-.370*	-.133 *-.103*	.043 *.001*	.039 *-.006*	.211 *.114*	.088 *.112*	.067 *.029*	.055 *.054*	.047 *.092*
0.005	-.049 *.689*	-.283 *-.595*	-.378 *-.491*	.367 *.444*	-.109 *-.354*	-.132 *-.106*	.055 *.002*	.043 *.008*	.211 *.108*	.092 *.113*	.063 *.045*	.052 *.053*	.059 *.084*
0.01	-.049 *.708*	-.314 *-.605*	-.416 *-.529*	.376 *.442*	-.117 *-.338*	-.123 *-.109*	.062 *.002*	.063 *.018*	.242 *.099*	.094 *.115*	.055 *.053*	.051 *.061*	.076 *.076*

[a]In the inner salt atom 9 moves to atom 1.
[b]Upper cases and lower cases in italics are related to non-dissociated acid and its inner salt, respectively

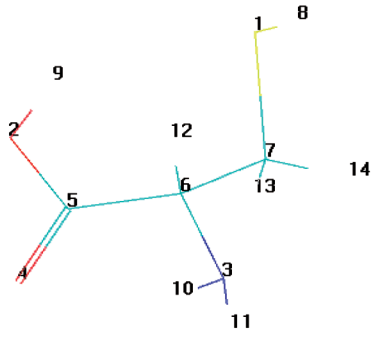

Figure 3
Atom Numbering in the Cys Molecule

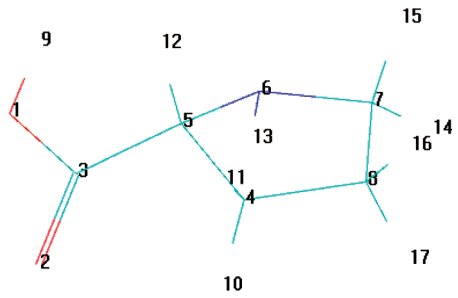

Figure 4
Atom Numbering in the Pro Molecule

Table 3
Charge Density at Particular Atoms of the Cys Molecule Varying With the EEF Strength[a,b,c]

EEF [a.u.]	Atoms													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
0.000	-.015 *-.184*	-.269 *-.291*	-.016 *1.159*	-.346 *-.345*	.323 *.635*	-.101 *-1.078*	-.181 *.054*	.010 *.003*	.220 *-.111*	.045 *-.071*	.041 *-.067*	.105 *.176*	.080 *.053*	.106 *.066*
0.001	-.015 *-.184*	-.272 *-.300*	-.014 *1.158*	-.353 *-.335*	.324 *.634*	-.100 *-1.078*	-.183 *.054*	.014 *.003*	.223 *-.107*	.044 *-.071*	.040 *-.064*	.106 *.175*	.079 *.052*	.107 *.063*
0.005	-.022 *-.228*	-.280 *-.313*	-.009 *1.159*	-.382 *-.315*	.333 *,629*	-.097 *-1.067*	-.191 *.078*	.038 *.008*	.231 *-.099*	.038 *-.074*	.038 *-.057*	.109 *.175*	.081 *.053*	.114 *.051*
0.01	-.036 *.048*	-.282 *-.345*	-.009 *1.140*	-.420 *-.340*	.345 *.683*	-.092 *-1.029*	-.202 *.124*	.069 *.029*	.242 *-086*	.031 *-.064*	.041 *-.036*	.108 *.178*	.086 *.050*	.124 *.048*

[a]In the inner salt atom 9 moves to atom 3.
[b]Upper cases and lower cases in italics are related to non-dissociated acid and its inner salt, respectively.
[c]The applied computation method indicated anomalously long bond between the carbonyl group and the α-carbon atom

Table 4
Charge Density at Particular Atoms of the Pro Molecule Varying With the EEF Strength[a,b]

EEF [a.u.]	Atoms								
	1	2	3	4	5	6	7	8	9
0.000	-.267 *-.585*	-.331 *-.457*	.330 *.444*	-.112 *-.086*	-.105 *-.328*	-.068 *.567*	-.084 *-.204*	-.116 *-.109*	.205 *.016*
0.001	-.265 *-589*	-.343 *-.465*	.337 *.443*	-.100 *-.085*	-.096 *-.325*	-.061 *.571*	-.085 *-.205*	-.108 *-.110*	.199 *.017*
0.005	-.276 *-.600*	-.364 *-.497*	,362 *.442*	-.113 *-.086*	-.106 *-.313*	-.106 *.587*	-.089 *-.208*	-.114 *-.112*	.207 *.023*
0.01	-.285 *-.610*	-.399 *-.537*	.377 *.441*	-.115 *-.085*	-.109 *-.301*	-.109 *.605*	-.089 *-.211*	-.117 *-.115*	.219 *.029*

EEF [a.u.]	Atoms							
	10	11	12	13	14	15	16	17
0.000	.082 *.094*	.065 *.075*	.088 *.109*	.055 *.141*	.058 *.100*	.063 *.100*	.060 *.062*	.058 *.080*
0.001	.061 *.092*	.065 *.076*	.090 *.109*	.073 *.138*	.050 *.100*	.062 *.086*	.057 *.081*	.065 *.065*
0.005	.060 *.083*	.069 *.078*	.096 *.110*	.061 *.131*	.055 *.101*	.070 *.098*	.079 *.077*	.061 *.085*
0.01	.058 *.080*	.079 *.073*	.100 *.110*	.049 *.120*	.057 *.120*	.074 *.100.*	.094 *.091*	.072 *.091*

[a]In the inner salt atom 9 moves to atom 6.
[b]Upper cases and lower cases in italics are related to non-dissociated acid and its inner salt, respectively.

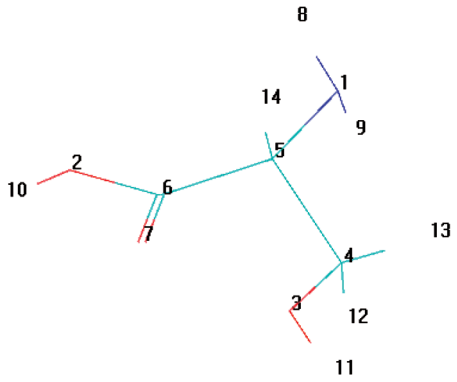

Figure 5
Atom Numbering in the Ser Molecule

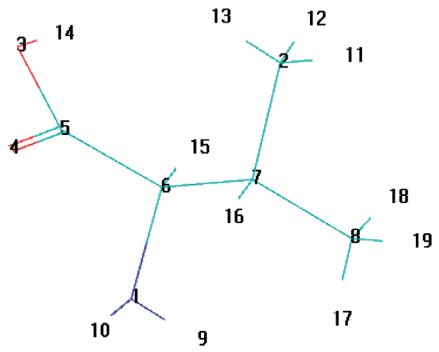

Figure 6
Atom Numbering in the Val Molecule

Table 5
Charge Density at Particular Atoms of the Ser Molecule Varying With the EEF Strength[a,b]

EEF [a.u.]	Atoms													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
0.000	-.021	-.288	-.311	.075	-.090	.353	-.402	.035	.040	.225	.194	.037	.044	.108
	.901	*-.486*	*-.313*	*.121*	*-.545*	*.489*	*-.589*	*.011*	*.018*	*-.039*	*.233*	*.042*	*.025*	*.133*
0.001	-.020	-.289	-.312	.076	-.090	.353	-.402	.033	.041	.224	.196	.038	.046	.107
	.897	*-.496*	*-.313*	*.121*	*-.545*	*,484*	*-591*	*.012*	*.018*	*-.034*	*.233*	*.042*	*.025.*	*.133*
0.005	-.011	-.281	-.316	.074	-.122	.362	-.420	.043	.034	.226	.199	.053	.052	.107
	.892	*-.537*	*-.355*	*.124*	*-.492*	*.472*	*-.574*	*.010*	*.023*	*-.016*	*.257*	*.041*	*.023*	*.131*
0.01	-.015	-.267	-.332	.077	-.122	.372	-.443	.058	.028	.226	.207	.041	.053	.118
	.888	*-.575*	*-.362*	*.117*	*-.461*	*.462*	*-.570*	*.013*	*.025*	*.001*	*.251*	*.056*	*.0.25*	*.130*

[a]In the inner salt atom 10 moves to atom 1.
[b]Upper cases and lower cases in italics are related to non-dissociated acid and its inner salt, respectively.

Table 6
Charge Density at Particular Atoms of the Val Molecule Varying With the EEF Strength[a,b]

EEF [a.u.]	Atoms									
	1	2	3	4	5	6	7	8	9	10
0.000	-.041	.119	-.264	-.338	.361	-.113	-.099	-.120	.043	.046
	.657	*-.122*	*-.453*	*-584*	*.446*	*-.373*	*-.054*	*-.134*	*.009*	*.116*
0.001	-.041	.119	-.160	-.333	.358	-.113	-.099	-.119	.041	.047
	.660	*-.122*	*-.461*	*-587*	*.445*	*-.370*	*.-054*	*-.135*	*.010*	*.115*
0.005	-.034	.122	-.282	-.282	.375	-.109	-.095	-.124	.039	.048
	.674	*-.121*	*-.492*	*-597*	*.442*	*-.354*	*-.055*	*-.140*	*.019*	*.108*
0.01	-.039	.129	-.298	-.298	.386	-.111	-.090	-.130	.034	.056
	.690	*-121*	*-.529*	*-609*	*.440*	*-.339*	*-.055*	*-.146*	*.030*	*.100*
EET [a.u.]	**Atoms**									
	11	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	
0.000	.050	.040	.053	.199	.079	.083	.042	.046	.051	
	.043	*.040*	*.091*	*-.003*	*.114*	*.082*	*.019*	*.046*	*.063*	
0.001	.048	.039	.056	.198	.078	.085	.041	.043	.049	
	.042	*.081*	*.089*	*-.001*	*.115*	*.081*	*.017*	*.021*	*.064*	
0.005	.050	.037	.057	.205	.076	.085	.041	.056	.061	
	.044	*.077*	*.078*	*.004*	*.116*	*.077*	*.030*	*.055*	*.070*	
0.01	.058	.027	.072	.215	.066	.095	.042	.066	.075	
	.045	*.064*	*.071*	*.011*	*.117*	*.071*	*.040*	*.065*	*.077*	

[a]In the inner salt atom 14 moves to atom 1.
[b]Upper cases and lower cases in italics are related to non-dissociated acid and its inner salt, respectively.

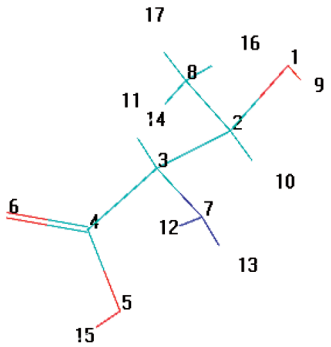

Figure 7
Atom Numbering in the Thr Molecule

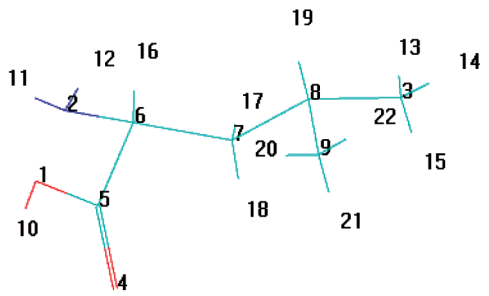

Figure 8
Atom Numbering in the Leu Molecule

Table 7
Charge Density at Particular Atoms of the Thr Molecule Varying With the EEF Strength[a,b]

EEF [a.u.]	Atoms																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
0.000	-.307	.100	-.124	.347	-.301	-.378	-.028	-.132	.201	.051	.109	.039	.040	.046	.231	.058	.057
	-.374	*.115*	*-.398*	.451	*-.583*	*-.460*	*.709*	*-.163*	*218*	*.065*	*.117*	*.104*	*.010*	*.105*	*-.010*	*.044*	*.049*
0.001	-.310	.100	-.124	.348	-.308	-.381	-.028	-.132	.203	.052	.107	.041	.042	.047	.233	.056	.055
	-.372	*.115*	*-.393*	*.450*	*-.586*	*-.467*	*.712*	*-.163*	*.220*	*.064*	*.117*	*.103*	*.012*	*.102*	*-.007*	*.044*	*.050*
0.005	-.321	.098	-.123	.352	-.294	-.398	-.031	-.130	.207	.062	.097	.044	.051	.051	.239	.051	.044
	-.364	*.115*	*-.376*	*.446*	*-.600*	*-.496*	*.724*	*-.163*	*.228*	*.062*	*.118*	*.095*	*.018*	*.090*	*.001*	*.048*	*.054*
0.01	-.334	.096	-.122	.358	-.273	-.425	-.034	-.127	.213	.076	.083	.047	.062	.057	.246	.045	.030
	-.333	*.110*	*-392*	*446*	*-.609*	*-.529*	*.727*	*-.158*	*.233*	*.076*	*.120*	*.090*	*.032*	*.075*	*.007*	*.056*	*.058*

[a]In the inner salt atom 15 moves to atom 7.
[b]Upper cases and lower cases in italics are related to non-dissociated acid and its inner salt, respectively.

Table 8
Charge Density at Particular Atoms of the Leu Molecule Varying With the EEF Strength[a,b]

EEF [a.u.]	Atoms										
	1	2	3	4	5	6	7	8	9	10	11
0.000	-.318	-.027	-.113	-.378	.381	-.027	-.114	-.076	.039	.227	.034
	-.474	*.864*	*-.116*	*-.598*	*.477*	*-.450*	*-.077*	*-.054*	*-.200*	*-.035*	*0.33*
0.001	-.299	-.030	-.114	-.401	.391	-.076	-.134	-.072	-.120	.222	.037
	-.482	*.862*	*-.117*	*-.601*	*.474*	*-.439*	*-.079*	*-.054*	*-200*	*-.031*	*.023*
0.005	-.297	-.038	-.118	-.421	.399	-.078	-.132	-.073	-.117	.213	.045
	-.514	*.857*	*-.118*	*-.604*	*.464*	*-.408*	*-.084*	*-.055*	*-200*	*-.016*	*.023*
0.01	-.293	-.050	-.123	-.448	.409	-.077	-.131	-.074	-.114	.204	.054
	-.550	*.855*	*-.120*	*-.606*	*.456*	*-.380*	*-.089*	*-.056*	*-.199*	*.002*	*.023*
EEF [a.u.]	**Atoms**										
	12	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
0.000	.036	.039	.042	.045	.069	.063	.088	.058	.039	.052	.042
	.016	*.047*	*.043*	*.027*	*.122*	*.025*	*.083*	*.052*	*.041*	*.139*	*.044*
0.001	.038	.042	.046	.042	.098	.060	.076	.066	.042	.045	.045
	.017	*.047*	*.043*	*.031*	*.122*	*.028*	*.082*	*.053*	*.040*	*.138*	*.044*
0.005	.035	.051	.056	.041	.104	.068	.069	.071	.030	.041	.051
	.018	*.046*	*.044*	*.040*	*.122*	*.042*	*.078*	*.058*	*.035*	*.135*	*.036*
0.01	.032	.062	.068	.043	.111	.079	.062	.075	.014	.037	.060
	.018	*.043*	*.038*	*.061*	*.123*	*.058*	*.072*	*.064*	*.028*	*.131*	*.028*

[a]In the inner salt atom 10 moves to atom 2.
[b]Upper cases and lower cases in italics are related to non-dissociated acid and its inner salt, respectively.

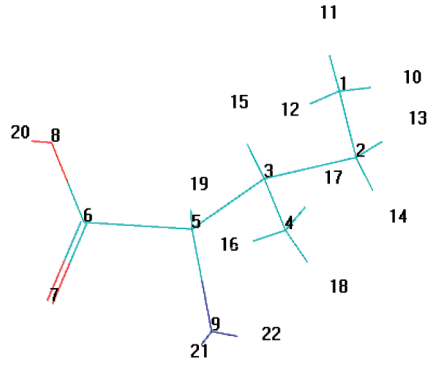

Figure 9
Atom Numbering in the Ile Molecule

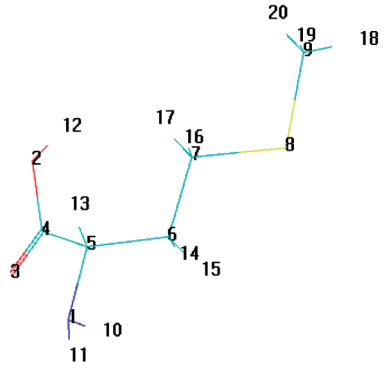

Figure 10
Atom Numbering in the Met Molecule

Table 9
Charge Density at Particular Atoms of the Ile Molecule Varying With the EEF Strength[a,b]

EEF [a.u.]	Atoms										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
0.000	-.115	-.110	-.067	-.123	-.087	.347	-.401	-.300	-.022	.043	.039
	-.117	*-.108*	*-.076*	*-.126*	*-.472*	*.481*	*-.604*	*-.477*	*.871*	*.057*	*.043*
0.001	-.116	-.099	-.068	-.123	-.088	.347	-.408	-.298	-.025	.040	.041
	-.117	*-.109*	*-.077*	*-127*	*-.460*	*.477*	*-.606*	*-.486*	*.869*	*.055*	*.037*
0.005	-.118	-.097	-.071	-.120	-.093	,356	-.435	-.277	-.023	.049	.055
	-.115	*-.115*	*-.080*	*-.129*	*-.425*	*.466*	*-.615*	*-.519*	*862*	*.048*	*.033*
0.01	-.120	-.096	-.075	-.118	-.097	.367	-.472	-.256	-.026	.057	.069
	-.113	*-.117*	*-.081*	*-.131*	*-.395*	*.457*	*-.622*	*-.555*	*.858*	*.038*	*.022*
EEF [a.u.]	**Atoms**										
	12	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
0.000	.042	.053	.067	.073	.048	.046	.050	.105	.227	.035	.042
	.038	*.065*	*.025*	*.125*	*-.074*	*.057*	*.016*	*.127*	*-.036*	*.014*	*.023*
0.001	.044	.051	.065	.075	.050	.045	.047	.105	.230	.036	.041
	.037	*.066*	*.028*	*.123*	*.073*	*.057*	*.019*	*.127*	*-.032*	*.015*	*.023*
0.005	.037	.060	.055	.087	.039	.054	.038	.115	.229	.028	.033
	.033	*.072*	*.040*	*.115*	*.065*	*.058*	*.030*	*.128*	*-.016*	*.015*	*.024*
0.01	.037	.073	.044	.100	.029	.069	.029	.122	.229	.017	.027
	.027	*.078*	*.054*	*.104*	*.058*	*.054*	*.043*	*.129*	*.002*	*.015*	*.023*

[a]In the inner salt atom 20 moves to atom 9.
[b]Upper cases and lower cases in italics are related to non-dissociated acid and its inner salt, respectively.

Table 10
Charge Density at Particular Atoms of the Met Molecule Varying With the EEF Strength[a,b]

EEF [a.u]	Atoms									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
0.000	-.018	-.273	-.320	.354	-.116	-.123	-.183	-.009	-.198	.034
	.877	*-.476*	*-.595*	*.484*	*-.479*	*-.065*	*-.193*	*-.032*	*-.196*	*.015*
0.001	-.017	-.275	-.326	.357	-.116	-.138	-.189	-.004	-.201	.034
	.688	*-.466*	*-.578*	*.449*	*-.383*	*-.071*	*-.194*	*-.026*	*-.198*	*.002*
0.005	-.018	-.290	-.351	.368	-.114	-.137	-.192	-.019	-.193	.039
	.698	*-.498*	*-.582*	*.446*	*-.370*	*-.075*	*-.193*	*-.014*	*-.204*	*.009*
0.01	-.025	-.304	-.386-*.583*	.381	-.113	-.139	-.193	-.047	-.179	.054
	.717	*-.534*		*.442*	*-.355*	*-.087*	*-185*	*-.030*	*-.210*	*.018*
EEF [a.u.]	**Atoms**									
	11	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
0.000	.048	.196	.087	.085	.078	.075	.063	.089	.065	.065
	.022	*-.037*	*.121*	*.077*	*.040*	*.128*	*.086*	*.082*	*.077*	*.065*
0.001	.046	.199	.088	.086	.068	.081	.084	.091	.064	.06
	.112	*-.004*	*.116*	*.075*	*.050*	*.125*	*.088*	*.081*	*.074*	*.065*
0.005	.044	.207	.092	.082	.076	.089	.073	.099	.068	.078
	.109	*.005*	*.117*	*.071*	*.062*	*.119*	*.094*	*.077*	*.064*	*.067*
0.01	.033	.218	.099	.076	.087	.101	.060	.105	.079	.088
	.102	*.015*	*.119*	*.071*	*.078*	*.111*	*.098*	*.081*	*.0.47*	*.081*

[a]In the inner salt atom 12 moves to atom 1.
[b]Upper cases and lower cases in italics are related to non-dissociated acid and its inner salt, respectively

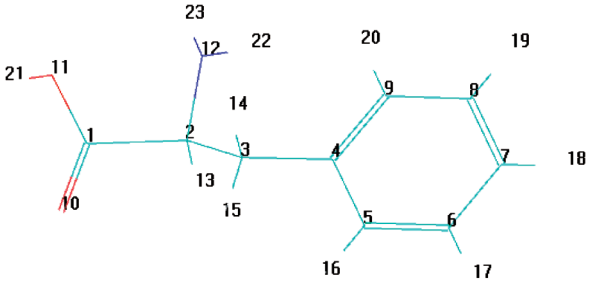

Figure 11
Atom Numbering in the Phe Molecule

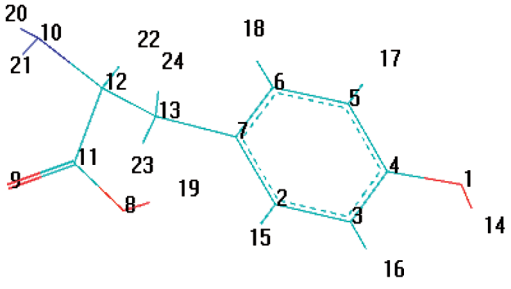

Figure 12
Atom Numbering in the Tyr Molecule

Table 11
Charge Density at Particular Atoms of the Phe Molecule Varying With the EEF Strength[a,b]

EEF [a.u]	Atom											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
0.000	.383	-.076	-.082	-.096	-.095	-.096	-.090	-.098	-.100	-.389	-.306	-.027
	.481	*-.458*	*-.048*	*-.126*	*-.093*	*-094*	*-.095*	*-.097*	*-.116*	*-.476*	*-.601*	*.867*
0.001	.384	-.076	-.082	-.097	-.097	-.098	-.097	-.095	-.098	-.395	-.302	-.030
	.476	*-.444*	*-.048*	*-.132*	*-.095*	*-.094*	*-.091*	*-.095*	*-.116*	*-.486*	*-.604*	*.864*
0.005	.393	-.074	-075	-.114	-.108	-.100	-.082	-.082	-.093	-.426	-.292	-.040
	.463	*-.405*	*-.045*	*-.155*	*-.102*	*-.090*	*-.073*	*-.086*	*-.117*	*-.519*	*-.615*	*.856*
0.1	.403	-.071	-.065	-.139	-,120	-.100	-.061	-.068	-.089	-.465	-.281	-.051
	.453	*-.372*	*-.037*	*-.184*	*-.109*	*-,084*	*-.050*	*-.076*	*-.120*	*-.556*	*-.626*	*.849*
EEF [a.u.]	**Atom**											
	13	**14**	15	16	17	18	19	20	21	22	23	
0.000	.099	.071	.072	.110	.106	.105	.105	.106	.228	.040	.037	
	.118	*.072*	*.114*	*.118*	*.109*	*.108*	*.108*	*.106*	*-.029*	*.020*	*.013*	
0.001	.097	.073	.071	.108	.104	.106	.108	.108	.228	.041	.039	
	.118	*.071*	*.111*	*.116*	*.110*	*.011*	*.110*	*.106*	*-.024*	*.020*	*.013*	
0.005	.093	.077	.065	.096	.103	.117	.121	.111	.219	.050	.043	
	.117	*.069*	*.102*	*.108*	*.115*	*.125*	*.120*	*.104*	*-.007*	*.021*	.013	
0.01	.090	.077	.057	.083	.105	.133	.136	.111	.206	.061	.048	
	.117	*.064*	*.090*	*.099*	*.123*	*.142*	*.132*	*.100*	*.010*	*.020*	*.013*	

[a]In the inner salt atom 21 moves to atom 12.
[b]Upper cases and lower cases in italics are related to non-dissociated acid and its inner salt, respectively.

Table 12
Charge Density at Particular Atoms of the Tyr Molecule Varying With the EEF Strength[a,b]

EEF [a.u.]	Atoms											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
0.000	-.222	-.048	-.189	.105	-.133	-.082	-.150	-.267	-.349	-.018	.324	-.121
	-.220	*-.068*	*-.195*	*.108*	*-.137*	*-.054*	*-.168*	*-.455*	*.584*	*.674*	*.447*	*-.374*
0.001	-.223	-.047	-.188	.105	-.134	-.083	-.151	-.271	-.353	-.015	.325	-.120
	-.218	*-.068*	*-.068*	*.113*	*-.138*	*-.054*	*-.173*	*-.464*	*.586*	*.678*	*.447*	*-.370*
0.005	-.216	-.083	-.140	.126	-.182	-.043	-.177	-.271	-.391	-.015	.337	-.123
	-.206	*-.058*	*-.142*	*.134*	*-.190*	*-0.64*	*-.199*	*-.497*	*.599*	*.691*	*.442*	*-353*
0.01	-.208	-.090	-.150	.151	-.172	-.034	-.207	-.280	-.435	-.019	.352	-.126
	-.190	*-.060*	*-.187*	*.162*	*-.148*	*-.062*	*-.223*	*-.536*	*.613*	*.707*	*.439*	*-.336*
EEF [a.u.]	**Atoms**											
	13	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
0.000	-.048	.200	.109	.115	.126	.113	.209	.042	.039	.095	.065	.084
	-.041	*.200*	*.106*	*.116*	*.128*	*.115*	*-.006*	*.112*	*.004*	*.114*	*.072*	*.107*
0.001	-.049	.201	.110	.116	.126	.112	.211	,041	.039	.095	.065	.087
	-.040	*.203*	*.104*	*.118*	*.128*	*.113*	*-.004*	*.111*	*.006*	*.114*	*.071*	*.105*
0.005	-.045	.214	.102	.127	.127	.110	.225	,034	.033	.101	.062	.090
	-.033	*.214*	*.104*	*.131*	*.128*	*.102*	*.001*	*.103*	*.015*	*.114*	*.095*	*.067*
0.01	-.039	.230	.088	.126	.142	.111	.241	.032	.022	.106	.052	.092
	-.022	*.230*	*.098*	*.140*	*.135*	*.092*	*.007*	*.092*	*.026*	*.115*	*.062*	*.082*

[a]Lower cases in italics are related to inner salt.
[b]In the inner salt atom 19 is attached to atom 10.

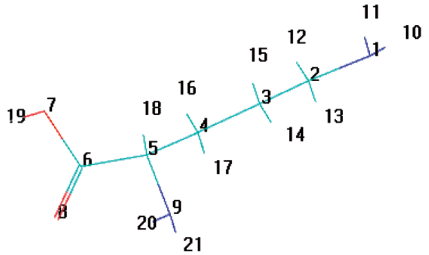

Figure 13
Atom Numbering in the Orn Molecule

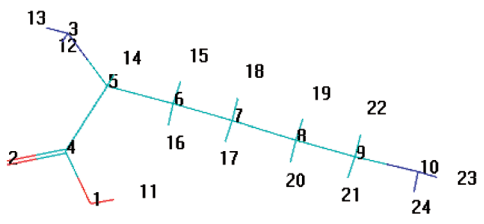

Figure 14
Atom Numbering in the Lys Molecule

Table 13
Charge Density at Particular Atoms of the Orn Molecule Varying With the EEF Strength[a,b]

EEF [a.u.]	Atoms										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
0.000	-.033	-.103	-.104	-.093	-.073	.342	-.299	-.397	-.022	.024	.023
	.829	*-.282*	*-.125*	*-.081*	*-.193*	*.422*	*-.604*	*-.595*	*-.086*	*.016*	*.017*
0.001	-.036	-.103	-.104	-.093	-.074	.342	-.295	-.400	-.023	.023	.025
	.830	*-.281*	*-.125*	*-.080*	*-.189*	*.421*	*-.605*	*-.601*	*-.084*	*.017*	*.018*
0.005	-.048	-.104	-.102	*-.094*	-.078	.349	-.277	-.422	-.029	.030	.029
	.836	*-.280*	*-.122*	*-.078*	*-.176*	*.417*	*-.610*	*-.626*	*-.074*	*.019*	*.021*
0.01	-.062	-.105	-.100	-.096	-.082	.358	-.254	-.450	-.036	.037	.035
	.842	*-.278*	*-.117*	*-.076*	*-.160*	*.413*	*-.616*	*-.658*	*-.062*	*.031*	*.024*
EEF [a.u.]	**Atoms**										
	12	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	
0.000	.026	.056	.080	.051	.059	.062	.097	.227	.036	.041	
	.125	*.122*	*.081*	*.067*	*.084*	*.056*	*.059*	*.004*	*.041*	*.042*	
0.001	.028	.054	.078	.053	.061	.060	.099	.229	.036	.039	
	.124	*.122*	*.082*	*.067*	*.083*	*.055*	*.060*	*-.007*	*.040*	*.040*	
0.005	.043	.048	.065	.059	.075	.053	.104	.235	.031	.032	
	.121	*.120*	*.083*	*.067*	*.081*	*.052*	*.060*	*.018*	*.037*	*.035*	
0.01	.061	.042	.050	.066	.090	.046	.112	.242	.025	.023	
	.118	*.118*	*.084*	*.118*	*.077*	*.051*	*.060*	*.022*	*.032*	*.028*	

[a]Lower cases in italics are related to inner salt.
[b]In the inner salt atom 19 is attached to atom 1.

Table 14
Charge Density at Particular Atoms of the Lys Molecule Varying With the EEF Strength[a,b]

EEF [a.u]	Atoms											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
0.000	-.270	-.344	-.018	.320	-.110	-.111	-.098	-.106	-.102	-.013	.204	.039
	-.590	*-.626*	*-.039*	*.400*	*-.120*	*-094*	*-.104*	*-.085*	*-.337*	*.651*	*-.014*	*.036*
0.001	-.269	-.351	-.017	.322	-.111	-.110	-.099	-.105	-.102	-.032	.206	.037
	-.590	*-.627*	*-.041*	*.400*	*-.122*	*-.094*	*-.104*	*-.086*	*-.337*	*.656*	*.011*	*.036*
0.005	.-268	-.385	-.013	.333	-.114	-.111	-.099	-.104	-.102	-.032	.218	.029
	-.586	*-.631*	*.-.051*	*.397*	*-.128*	*-.094*	*-.104*	*-.089*	*-.336*	*.676*	*.001*	*.036*
0.01	-.283	-.425	.001	.346	-.112	-.113	-.096	-.106	-.101	-.050	.231	.026
	-.585	*-.634*	*-.067*	*.396*	*-.135*	*-.095*	*-.104*	*-.096*	*-.333*	*.697*	*.012*	*.018*
EEF [a.u.]	**Atoms**											
	13	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**14**
0.000	.042	.089	.083	.063	.035	.060	.071	.053	.026	.057	.026	.024
	.031	*.083*	*.059*	*.056*	*.049*	*.078*	*.052*	*.060*	*.213*	*.080*	*.012*	*.150*
0.001	.041	.092	.082	.061	.035	.062	.069	.051	.027	.059	.029	.022
	.029	*.081*	*.057*	*.057*	*.050*	*.078*	*.055*	*.060*	*.211*	*.083*	*.012*	*.147*
0.005	.034	.100	.084	.055	.032	.062	.068	.047	.030	.062	.043	.021
	.022	*.075*	*.051*	*.059*	*.076*	*.053*	*.068*	*.061*	*.204*	*.062*	*.014*	*.135*
0.01	.025	.108	.095	.049	.023	.079	.066	.049	.043	.055	.039	.050
	.037	*.066*	*.046*	*.063*	*.071*	*.057*	*.084*	*.068*	*.193*	*.106*	*.017*	*.118*

[a]Lower cases in italics are related to inner salt.
[b]In the inner salt atom 11 is attached to atom 10.

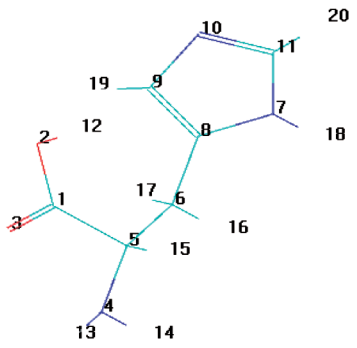

Figure 15
Atom Numbering in the His Molecule

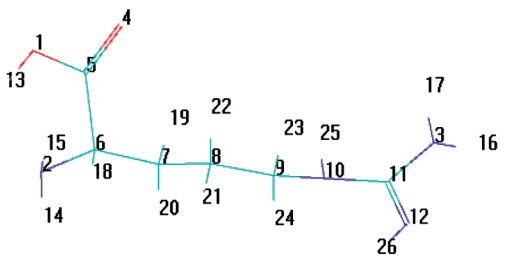

Figure 16
Atom Numbering in the Arg Molecule

Table 15
Charge Density at Particular Atoms of the His Molecule Varying With the EEF Strength[a,b]

EEF [a.u.]	Atoms									
	1	2	3	4	5	6	7	8	9	10
0.000	.359	-.254	-.340	-.030	-.122	-.004	.326	-.340	-.109	-.111
	.523	*-.253*	*-.270*	*.045*	*-.100*	*-.013*	*-.115*	*-.120*	*-.303*	*.307*
0.001	.361	-.259	-.342	-.031	-.121	-.005	.331	-.337	-.111	-.116
	.523	*-.254*	*-.269*	*.045*	*-.101*	*-.012*	*-.122*	*-.123*	*-.301*	*.310*
0.005	.366	-.276	-.360	-.045	-.118	-.002	.345	-.328	-.118	-.140
	.525	*-.254*	*-.270*	*.037*	*-.101*	*-.005*	*-.145*	*-.135*	*-.285*	*.332*
0.01	.381	-.285	-.403	-.050	-.113	-.007	.369	-.332	-.142	-.169
	.517	*-.334*	*-.183*	*.085*	*-.089*	*-.025*	*-.174*	*-.170*	*-.285*	*.372*
EEF [a.u.]	**Atoms**									
	11	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
0.000	-.242	.206	.045	.034	.068	.065	.069	.070	.144	.166
	-.261	*.069*	*.024*	*.025*	*.027*	*.052*	*.067*	*-.013*	*.163*	*.161*
0.001	-.244	.206	.045	.037	.071	.067	.068	.072	.143	.166
	-.264	*.070*	*.027*	*.026*	*.026*	*.053*	*.064*	*.068*	*.171*	*.159*
0.005	-.245	.216	.044	.053	.083	.069	.070	.080	.140	.166
	-.276	*.078*	*.026*	*.036*	*.017*	*.050*	*.049*	*.061*	*.181*	*.155*
0.01	-.240	.225	.049	.066	.090	.082	.068	.093	.137	.171
	-.284	*.097*	*.020*	*.054*	*.026*	*.061*	*.048*	*.061*	*.170*	*.168*

[a]Lower cases in italics are related to inner salt
[b]In the inner salt atom 12 is attached to atom 10.

Table 16
Charge Density at Particular Atoms of the Arg Molecule Varying With the EEF Strength[a,b]

EEF [a.u.]	Atoms												
	1	2	3	4	5	6	7	8	9	10	11	12	13
0.000	-.263	-.039	.046	-.342	.358	-.119	-.116	-.108	-.089	-.004	-.100	-.204	.208
	-.496	*-.044*	*.111*	*-.644*	*.401*	*-.154*	*-.097*	*-.110*	*-.083*	*.056*	*-.103*	*.093*	*.062*
0.001	-.263	-.039	.038	-.348	.360	-.119	-.115	-.109	-.086	-.017	-.106	-.183	.211
	-.611	*-.042*	*.131*	*-.596*	*.394*	*-.125*	*-.094*	*-.125*	*-.072*	*-.024*	*-.067*	*.101*	*.072*
0.005	-.269	-.038	.019	-.381	.370	-.117	-.113	-.110	-.082	-.043	-.116	-.150	.223
	-.614	*-.051*	*.123*	*-.596*	*.392*	*-.130*	*-.094*	*-,124*	*-.072*	*-.022*	*-.070*	*.127*	*.081*
0.01	-.315	-.049	.050	-.408	.377	-.122	-.104	-.108	-.093	-.004	-.071	-.285	.241
	-.616	*-.067*	*.107*	*-.597*	*.391*	*-.135*	*-.095*	*-.121*	*-.076*	*-.032*	*-.076*	*.179*	*.095*
EEF [a.u]	**Atoms**												
	14	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**
0.000	.041	.040	.058	.029	.089	.075	.055	.063	.071	.047	.059	.067	.080
	.038	*.036*	*.121*	*.059*	*.070*	*.058*	*.068*	*.061*	*.074*	*.051*	*.067*	*.243*	*.062*
0.001	.043	.041	.055	.030	.089	.074	.056	.062	.073	.044	.059	.068	.084
	.035	*.031*	*.209*	*.060*	*.082*	*.050*	*.061*	*.057*	*.092*	*.059*	*.060*	*.199*	*.061*
0.005	.054	.044	.051	.036	.092	.068	.065	.063	.072	.038	.068	.064	.092
	.036	*.025*	*.198*	*.065*	*.073*	*.055*	*.057*	*.059*	*.082*	*.065*	*.069*	*.191*	*.071*
0.01	.062	.062	.044	.047	.097	.068	.080	.061	.065	.082	.059	.077	.080
	.038	*.023*	*.185*	*.072*	*.063*	*.061*	*.052*	*.061*	*.077*	*.071*	*.077*	*.179*	*.086*

[a]Lower cases in italics are related to inner salt.
[b]In the inner salt atom 13 is attached to atom 12.

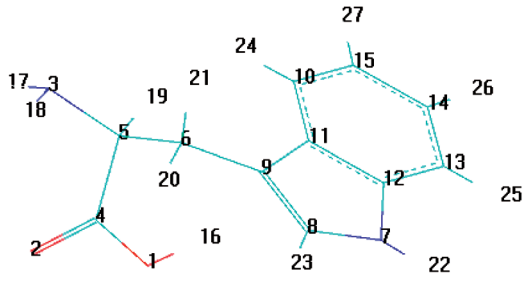

Figure 17
Atom Numbering in the Trp Molecule

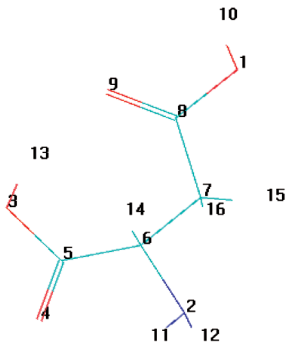

Figure 18
Atom Numbering in the Asp Molecule

Table 17
Charge Density at Particular Atoms of the Trp Molecule Varying With the EEF Strength[a,b]

EEF [a.u.]	Atoms													
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
0.000	-.270 *-.447*	-.356 *-.591*	-.021 *.664*	,329 *.446*	-.126 *-.387*	-.013 *.019*	.291 *258.*	-.212 *-.180*	-.176 *-.144*	-.067 *-.071*	-.090 *-.064*	-.171 *-.158*	-.093 *-086*	-.085 *-.094*
0.001	-.270 *-.468*	-.364 *-.592*	-.021 *.669*	.332 *-444.*	-.127 *-.361*	-.009 *-.009*	.307 *.290*	-.215 *-.201*	-.186 *-.202*	-.071 *-.082*	-.090 *-.092*	-.175 *-.171*	-.089 *-.085*	-.085 *-.111*
0.005	-.269 *-.498*	-.400 *-.600*	-.021 *.678*	.343 *-.440*	-.133 *-.343*	-.003 *-.001*	.325 *.330*	-.209 *-.207*	-.219 *-.236*	-.086 *-.102*	-.085 *-.095*	-.177 *-.179.*	-.072 *-.068*	-.084 *-.081*
0.01	-.260 *-.535*	-.444 *-.626*	-.024 *.691*	.358 *.437*	-.139 *-.326*	.007 *.015*	.360 *.360*	-.198 *-.204*	-.265 *-.276*	-.109 *-.131*	-.076 *-.095*	-.183 *-.182*	-.045 *-.045*	-.089 *-.078*

EEF [a.u.]	Atoms												
	15	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**
0.000	-.116 *-.115*	.214 *-.008*	.040 *-.002*	.039 *.118*	.091 *.131*	.060 *.069*	.078 *.031*	0.72 *.071*	.145 *.143*	.113 *.088*	.109 *.107*	.103 *.101*	.105 *.100*
0.001	-.114 *-.115*	.217 *-.005*	.038 *.018*	.037 *108*	.101 *.109*	.058 *.098*	.078 *.073*	.074 *.076*	.145 *.148*	.110 *.105*	.111 *.113*	.105 *.108*	.104 *.106*
0.005	-.109 *-.100*	.230 *.,002*	.030 *.031*	.030 *.100*	.110 *.109*	.053 *.087*	.080 *.030*	.084 *.083*	.145 *.144*	.100 *.097*	.123 *.126*	.113 *.108*	.100 *105*
0.01	.-103 *-.087*	.246 *.006*	.020 *.045*	.025 *.088*	.118 *.076*	.048 *.076*	.080 *.109*	.095 *.093*	.147 *.141*	.086 *.086*	.130 *.142*	.122 *.133*	.092 *.102*

[a]Lower cases in italics are related to inner salt
[b]In the inner salt atom 16 is attached to atom 3.

Table 18
Charge Density at Particular Atoms of the Asp Molecule Varying With the EEF Strength[a],[b]

EEF [a.u.]	Atoms															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
0.000	-.284 *-.346*	-.021 *1.183*	-.288 *.241*	-.355 *-.298*	.326 *.540*	-.111 *-1.105*	-.104 *.110*	.395 *.349*	-.423 *-.406*	.236 *.221*	.046 *-.073*	.043 *-.085*	.243 *-.128*	.095 *.177*	.108 *.069*	.093 .032
0.001	-.279 *-.341*	-.018 *1.181*	-.292 *.249*	-.362 *-.291*	.327 *.541*	-.110 *-1.106*	-.105 *.111*	.396 *.350*	-.427 *-.414*	.237 *.221*	.045 *-.071*	.042 *-.083*	.245 *-.125*	.096 *.176*	.111 *.067*	.093 *.032*
0.005	-.259 *-.321*	-.009 *1.175*	-.305 *.276*	-.391 *-.263*	.336 *.540*	-.107 *-1.109*	-.109 *.118*	.400 *.356*	-.442 *-.448*	.245 *.217*	.039 *-0.61*	.038 *-.078*	.254 *-.113*	.099 *.173*	.121 *.055*	.089 *.035*
0.01	-.234 *-.296*	.000 *1.167*	-.320 *.314*	-.429 *-.222*	.348 *.537*	-.103 *-1.111*	-.114 *.126*	.406 *.362*	-.462 *-.491*	.255 *.210*	.030 *-.049*	.036 *-.070*	.266 *-.099*	.102 *.169*	.132 *.042*	.087 *.040*

[a]Lower cases in italics are related to inner salt.
[b]In the inner salt atom 13 is attached to atom 2.

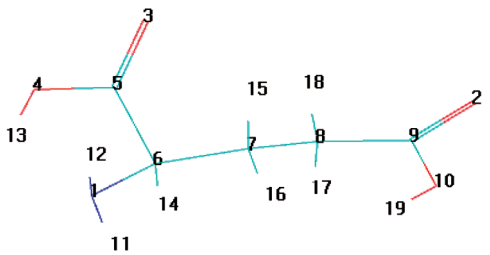

Figure 19
Atom Numbering in the Glu Acid Molecule

Table 19
Charge Density at Particular Atoms of the Glu Acid Molecule Varying With the EEF Strength[a,b]

EEF [a.u.]	Atoms									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
0.000	-.035 *1.151*	-.325 *-356*	-.333 *-.284*	-.258 *-.396*	.355 *.486*	-.120 *-.998*	-.114 *.073*	-.142 *-.155*	.351 *.358*	-.272 *-.271*
0.001	-.034 *1.151*	-.332 *-.366*	-.341 *-.280*	-.257 *-.418*	.358 *.491*	-.120 *-1.001*	-.113 *.072*	-.142 *.-.155*	.354 *.364*	-.272 *-.282*
0.005	-.029 *1.132*	-.362 *-.408*	-.374 *-.268*	-.259 *-.454*	.367 *.501*	-.119 *-.984*	-.111 *.070*	-.145 *-.153*	.364 *.376*	-.270 *-.325*
0.01	-.031 *1.116*	-.395 *-.439*	-.408 *-.265*	-.270 *-.493*	.376 *.505*	-.117 -.968	-.105 *.072*	-.141 *-.150*	.377 *.387*	-.271 *-.347*
EEF [a.u.]	**Atoms**									
	11	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	
0.000	.040 *-.073*	.042 *-.086*	.209 *-.062*	.089 *.155*	.086 *.071*	.045 *.030*	.076 *.062*	.111 *.089*	.194 *.207*	
0.001	.042 *-.067*	.042 *-.086*	.212 *-.061*	.090 *.159*	.084 *.069*	.046 *.030*	.077 *.068*	.110 *.083*	.196 *.228*	
0.005	.053 *-.054*	.042 *-.066*	.224 *-.050*	.095 *.170*	.077 *.062*	.053 *.034*	.082 *.074*	.106 *.079*	.206 *.264*	
0.01	.069 *-.042*	.044 *-.049*	.235 *-.039*	.101 *.181*	.069 *.052*	.069 *.041*	.087 *.078*	.096 *.085*	.217 *.274*	

[a]Lower cases in italics are related to inner salt
[b]In the inner salt atom 13 is attached to atom 1.